EVALUATION OF PHARMACOLOGICAL EFFECT OF APOLIPOPROTEIN NANOPARTICLES OF NORETHISTERONE FOR ANOVULATION INFERTILITY AND FUNCTIONAL HYPOTHALAMIC AMENORRHEA ASSOCIATED WITH POLYCYSTIC OVARY SYNDROME SYMPTOMS IN FEMALE RATS

SYNOPSIS OF THE THESIS

Submitted by

Shalini Jamwal

Registration No. AU20PYPhD0043

Under supervision of

Dr. Abhishek Soni

In Partial Fulfillment of the Requirement

for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF PHARMACY

ABHILASHI UNIVERSITY

CHAIL CHOWK, MANDI (H.P.) – 175028

DECLARATION

I hereby declare that the dissertation synopsis entitled **"Evaluation of pharmacological effect of apolipoprotein nanoparticles of norethisterone for anovulation infertility and functional hypothalamic amenorrhea associated with Polycystic ovary syndrome symptoms in female rats"** to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles. It has not been submitted to any otherUniversity or Institution for the award of any degree or diploma.

Place: Chail Chowk

Date: 4/02/2022

Signature of the Scholar

Shalini Jamwal

Forwarded by: Concern Supervisor

Place: Chail Chowk

Date: 4/2/22

Signature of the Supervisor

Dr. Abhishek Soni

Forwarded

Dean
School of Pharmacy
Abhilashi University
Chali-Chowk, Distt. Mandi (H.P.)
04/02/2022

1. Introduction:

1) Anovulation

Anovulation is a common cause of infertility. It is caused by hormonal imbalances, and the main sign of it is having irregular periods. Anovulation can often be treated with lifestyle changes and/or medication.

Anovulation happens when an egg (ovum) doesn't release from your ovary during your menstrual cycle. An egg is needed to have a pregnancy. Since multiple hormones are involved in ovulation, there are many which causes the anovulation. Chronic anovulation is a common cause of infertility.

Ovulation is the release of an egg (ovum) from your ovary. Ovulation is part of the menstrual cycle. On average, it happens on day 14 of a 28-day menstrual cycle. There are multiple hormones involved in ovulation.

The process of ovulation begins when your hypothalamus (a part of your brain) releases gonadotropin-releasing hormone (GnRH). GnRH causes your pituitary gland (a gland in your brain) to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

Between days six and 14 of the menstrual cycle, FSH causes follicles (small sacs of fluid in the ovaries that contain a developing egg) in one ovary to begin to mature. During days 10 to 14 of the cycle, only one of the developing follicles forms a fully mature egg. Around day 14 in the menstrual cycle, a sudden surge in LH causes the ovary to release its egg. This is called ovulation. After ovulation, the egg begins its five-day journey through your fallopian tube to your uterus. As the egg travels through your fallopian tube, the level of progesterone, another hormone, in your body rises, which helps prepare your uterine lining for pregnancy.

Signs and symptoms of anovulation:

- **Having irregular periods:** If the length of time in between your periods keeps changing, it's considered an irregular period. The average menstrual cycle is 28 days, but it can be a couple of days shorter or longer than that.

- **Having very heavy or light periods:** A heavy period is defined by losing over 16 teaspoons (80 mL) of blood within your period and/or having a period that lasts longer than seven days. Blood loss of fewer than four

teaspoons (20 mL) throughout your period is considered a light period.

• **Having a lack of periods (amenorrhea):** Missing one or more periods without being pregnant could be a sign of anovulation.

• **Having a lack of cervical mucus:** Right before and during ovulation, you usually have the most vaginal discharge called cervical mucus. It usually looks like raw egg whites. If you don't have this discharge, you may be experiencing anovulation.

Causes of anovulation:

In general, the cause of anovulation is an imbalance of one or more certain hormones, especially the hormones involved in ovulation, which include:

• Gonadotropin-releasing hormone (GnRH).

• Follicle-stimulating hormone (FSH).

• Luteinizing hormone (LH).

Anovulation treatment:

Treatment for anovulation depends on correcting the hormonal imbalance that's causing it, if possible.

In general, lifestyle changes that may treat your anovulation include:

• **Managing your stress:** If your healthcare provider suspects that stress could be causing your anovulation, they'll likely recommend trying to limit your stressors and using techniques such as meditation, yoga or deep breathing to better cope with stress.

• **Managing your weight:** If you have obesity, your healthcare provider may recommend trying to lose weight to treat your anovulation. If you have a low body mass index (BMI) your healthcare provider may recommend gaining weight to treat your anovulation. If either of these situations applies to you, be sure to talk to your provider and/or a registered dietitian to manage your weight safely and in a way that works for you.

• **Decreasing your exercise frequency and intensity:** If your healthcare provider suspects that your exercise routine and intensity are causing your anovulation, they may recommend limiting the amount of time you exercise

and the intensity of the exercises.

Other treatments for anovulation include:

• **Certain medications to treat other conditions:** If you have a condition that's causing your anovulation that's treatable, your healthcare will likely prescribe you medication, which could also treat your anovulation. For example, if you have hypothyroidism, which can cause anovulation, taking medication to treat your hypothyroidism could also treat your anovulation.

• **Adjusting current medications:** Some medications can cause anovulation, such as anti-epileptic drugs and antipsychotic drugs. If you're trying to conceive, your healthcare provider might adjust your current medications to try to treat your anovulation, if possible. Never adjust or stop taking your medications without seeing your healthcare provider. Always follow the instructions on your prescribed medications.

• **Clomiphene citrate (CC):** This medication helps correct ovulatory irregularities.

• **Human chorionic gonadotropin (hCG) injection:** This hormone causes your ovary to release an egg. A synthetic form of it can be injected to help treat anovulation. It's often taken with clomiphene citrate.

• **Follicle-stimulating hormone (FSH) injection:** If your body isn't making enough FSH and other treatments for anovulation haven't worked, your healthcare provider may have you get an injection of synthetic FSH to help your ovary release an egg.

• **Gonadotropin-releasing hormone (GnRH) agonists and antagonists injections:** An injection of GnRH agonists and antagonists helps to control the levels of luteinizing hormone (LH) your body makes, which is needed for ovulation.

2) Functional hypothalamic amenorrhea:

Functional hypothalamic amenorrhea (FHA) is one of the most common causes of secondary amenorrhea. There are three types of FHA: weight loss-related, stress-related, and exercise-related amenorrhea. FHA results from the aberrations in pulsatile gonadotropin-releasing hormone (GnRH) secretion, which in turn causes impairment of the gonadotropins (follicle-stimulating

hormone and luteinizing hormone)[1]. The final consequences are complex hormonal changes manifested by profound hypoestrogenism. Additionally, these patients present mild hypercortisolemia, low serum insulin levels, low insulin-like growth factor 1 (IGF-1) and low total triiodothyronine. Functional hypothalamic amenorrhea (FHA) is classified as hypogonadotropic hypogonadism related to an aberration of the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus [2, 3]. The spectrum of hypothalamic–pituitary disturbances in FHA may be very broad and includes a lower mean frequency of LH pulses, the complete absence of LH pulsatility, as well as a normal-appearing secretion pattern and higher mean frequency of LH pulse [4]. In turn, decreased gonadotropin secretion leads to reduced estradiol production in the ovary. The disturbed hypothalamic–pituitary-ovarian axis in FHA cases is associated typically with stress, weight loss and/or excessive physical exercise and is one of the most common causes of secondary amenorrhea. Depending on the eliciting factor, there are three classes of FHA: weight loss related, stress related and exercise related [5]. Functional hypothalamic amenorrhea is related to profound impairment of reproductive functions including anovulation and infertility.

Regardless of the specific trigger, a complex state of hypoestrogenism, other endocrinological aberrations and metabolic abnormalities due to FHA may impact the whole body homeostasis [6].

Hormonal disturbances associated with FHA:

As described above, FHA results from depression of the hypothalamic–pituitary–ovarian axis. Impairment of GnRH and gonadotrophin secretion as a sequel is the key pathology in this trait, and also other pituitary hormone secretion is abnormal in FHA.

A typical feature of FHA is hypothalamic–pituitary–adrenal axis activation, related to stressing factors, and this phenomenon is believed to be one of the important pathogenetic factors in FHA patients [7]. Increased corticotropin-releasing hormone (CRH) secretion results in an increased secretion of adrenocorticotrophin from the pituitary and cortisol from the adrenal glands, and these phenomena are linked to a reduced GnRH drive. Elevated serum

and also cerebrospinal cortisol concentrations have been reported in FHA

Disturbances in the hypothalamic–pituitary–thyroid axis in FHA patients are also observed [8]. They include a low-to-normal level of thyrotropin, an increased level of reverse triiodothyronine and a low level of triiodothyronine [9].

A regular menstrual cycle is a marker of physical and mental well-being in all the women of reproductive age. Multiple factors can act as disruptors of the hypothalamic–pituitary–ovarian (HPO) axis physiology and cause menstrual disorders [10]. According to the most recent Endocrine Society Guidelines, functional hypothalamic amenorrhea (FHA) is defned as "a form of chronic anovulation that is not due to identifable organic causes", which occurs when disrupted gonadotropin-releasing hormone (GnRH) drive reduces both LH and FSH pulse with a consequent impairment of follicular development and ovulatory function. Psychological stress, nutritional deprivation and strenuous exercise training are the most widely studied factors known so far [11].

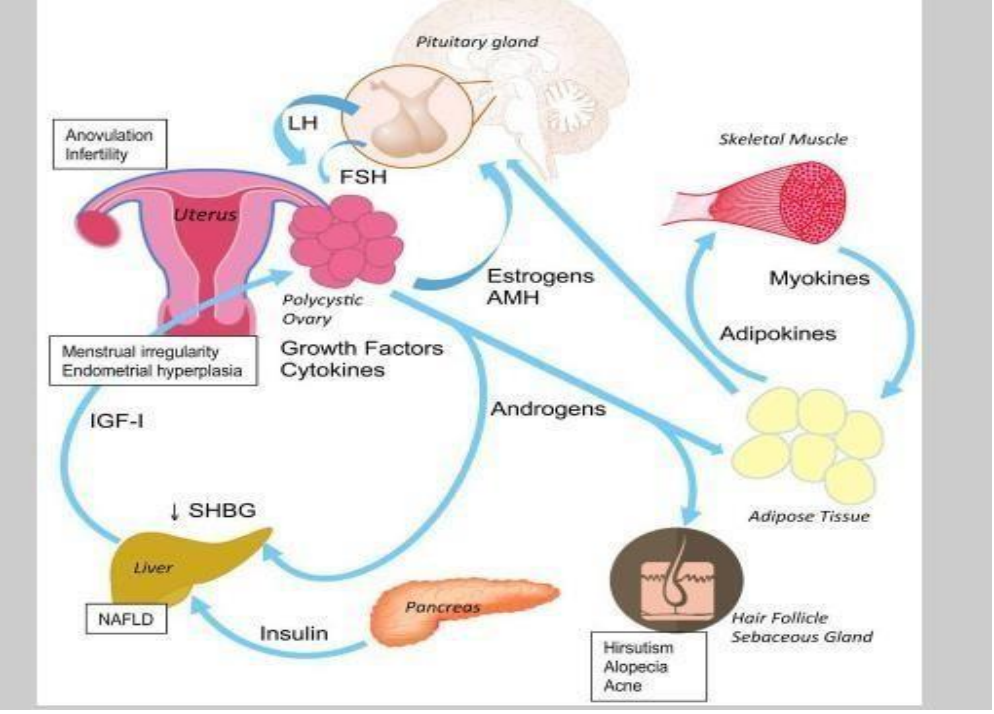
FHA is due to the activation of nervous, metabolic and hormonal pathways that lead to a reduction of GnRH pulsatile secretion by hypothalamic neurons [12, 13]. Two main biological systems are involved in stress response: the sympathetic adreno-medullary axis and the hypothalamic–pituitary–adrenal (HPA) axis that are activated by acute and chronic stresses, respectively [14]. The activation of the HPA axis increases secretion of the corticotrophin-releasing factor (CRH) and, consequently, of endogenous opioid peptides and endorphins which impair pulsatile GnRH release[15]. As expected,exogenous administration of opiates inhibits the pulsatile secretion of gonadotropins in humans and animals, while their antagonists naloxone and naltrexone restore the pulsatile secretion of GnRH and increase the levels of LH [16].

(LH) play a crucial role in controlling reproductive function in females and males. The pleiotropic effects of gonadotropins are manifested on various cells of the reproductive system including LH and FSH on ovarian granulosa cells, LH on theca interna cells, FSH on testicular Sertoli cells, and LH on Leydig cells [17].

3) PCOS:

Polycystic ovary syndrome (PCOS) is a prevalent, complex endocrine disorder characterized by polycystic ovaries, chronic anovulation and hyperandrogenism leading to symptoms of irregular menstrual cycles, hirsutism, acne and infertility. Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in premenopausal women. Heterogeneous by nature, PCOS is defined by a combination of signs and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnoses [18]. The etiology of this syndrome remains largely unknown, but mounting evidence suggests that PCOS might be a complex multigenic disorder with strong epigenetic and environmental influences, including diet and lifestyle factors. PCOS is frequently associated with abdominal adiposity, insulin resistance, obesity, metabolic disorders and cardiovascular risk factors [19]. The diagnosis and treatment of PCOS are not complicated, requiring only the judicious application of a few well-standardized diagnostic methods and appropriate therapeutic approaches addressing hyperandrogenism, the consequences of ovarian dysfunction and the associated metabolic disorders [20, 21]. This article aims to provide a balanced review of the latest advances and current limitations in our knowledge about PCOS while also providing a few clear and simple principles, based on current evidence-based clinical guidelines, for the proper diagnosis and long-term clinical management of women with PCOS [22].

Pathologic Process: An organic process occurring as a consequence polycystic ovary syndrome is still lacking because of the heterogeneity/assortment/heterogeneousness of this syndrome [23]. There are several theories have been proposed to explain the pathologic process of polycystic ovary syndrome (Fig1.) [24,25].



Physiological Sign and Symptom:

Acne

The features of acne include comedowns and pustules, which usually form during puberty but can continue into adulthood. They vary in severity and location [26, 27].

Hirsutism: Hirsutism is defined as male-pattern hair distribution occurring in women.This symptom is often distressing and results from hyperandrogenism. [28].

Oligomenorrhea and amenorrhoea: Oligomenorrhoea is characterised by infrequent ovulation with menses occurring more than 35 days apart. [29].

Oligoovulation: is infrequent or irregular ovulation, usually as cycles of > 36 days or < 8 cycles per year. Amenorrheic is the absence of a period. [30].

Amenorrhea: is the absence of a period. Usually, this problem occurs as either temporary or permanent ending of periods in a woman, who has menstruated in the past [31].

Treatment

1. Metformine: lowers insulin levels. It can help with weight loss and may prevent you from getting type 2 diabetes. It may also make the patient more

fertile.

2. Clomiphene citrate is a non-steroidal compound. Clomiphene citrate has been used for induction of ovulation since 1962. It is the treatment of first choice in women with ovulatory disorders who are normally oestrogenic, i.e. predominantly those with polycystic ovaries (PCOS) [32].

3. Letrozole, in undesirable anti-estrogenic features of this agent on endometrium and cervical mucus, Ovulatory Hyper Stimulation Syndrome and multifetal pregnancy have forced investigators to look forward to another drug with minor side-effects and acceptable efficacy. Letrozole is an aromatase inhibitor with less anti-estrogenic properties. Also there is no need for closed monitoring during therapy with Letrozole [33].

7. Rosiglitazone, Pioglitazone are an oral drug that reduces the amount of sugar (glucose) in the blood. It is used for treating patients with type 2 diabetes. It may also make the patient more fertile [34].

11. Ayurvedic treatment: PCOS can also cured successfully by using the Ayurveda treatment regimen. In Ayurveda different drugs as Triphala Kwatha, Chandraprabhaand, Manibhadra, (Asparagus racemosus), Shatapushpa (Peucedanum gravel oens) and Guduchi (Tinospora cordifolia), Terminalia chebula, T. belearica, Embelica officinalae, are used to treat PCOS [35].

- **Purpose of study:**
- Evaluation of pharmacological effect of apolipoprotein nanoparticles of norethisterone for anovulation infertility and functional hypothalamic amenorrhea associated with PCOS symptoms in female rats.

- **Background of topic**: Norethisterone and its acetate are progestogens. Norethisterone was the first orally active progestogen synthesized, and as such, it has been referred to as a first generation progestogen. Norethisterone is derived from nortestosterone. Norethisterone exerts its effects by binding to steroid receptors in the reproductive tract, breast, liver, and in the brain. The affinity of norethisterone for endometrial progesterone receptors is similar to that of the natural ligand and so it can produce secretory changes in the estrogen-primed endometrium. Like endogenous progesterone, in the second half of the menstrual cycle, norethisterone also exerts a central thermogenic effect. However, in some animal tests for progestogenic activity norethisterone acts as a competitive antagonist to progesterone. Norethisterone can influence reproductive behaviors, during estrus and mating in animals. Norethisterone is most commonly used in combined oral contraceptives or alone. Norethisterone and norethisterone acetate are used in hormone replacement regimens to oppose the effects of estrogen on the endometrium [36].

- **Drug profile:**

Chemical Names:	Norethindrone; Norethisterone
Chemical Formula:	C_{20} H_{26} O_2
Properties:	White/cream odorless crystalline powder; protect from light.
Molecular Weight:	298.4
Solubility:	Practically insoluble in water; slightly soluble in ethanol and ether. Soluble in chloroform and dioxan.

DESCRIPTION:

Pharmacokinetics:

- Norethisterone is rapidly and completely absorbed after oral administration. Peak plasma levels generally are attained after 1–3 hrs. When the enanthate is used parenterally (i.m.) uptake from the injection site is rapid; peak plasma concentrations are reached within 8 days.

Absorption:	Oral absorption 100%
Volume of Distribution:	240
Plasma Protein Binding:	61 %(albumin)
Plasma Half-Life:	7-8hrs

Pharmacodynamics:

- In the hypothalamus and pituitary gland, norethisterone depresses luteinizing Hormone production and secretion, but only inhibits ovulation when administered orally at daily doses of 5–10mg. Large parenteral doses can suppress ovulation. In the ovary, norethisterone has little effect on corpus luteum function in small doses, but in higher doses it can impair steroidogenesis. In the estrogen-primed (estrogen priming. is necessary to induce progesterone receptor formation) endometrium, norethisterone produces secretory changes leading to atrophic change and reduced mucus secretion. In the Fallopian tubes norethisterone can increase the frequency, but decreases the amplitude of smooth muscle contractions, as well as reducing the height and numbers of the cilia lining the tube. In the mucus secreted from the cervix, norethisterone increases viscosity and reduces Elasticity.

.

2. Review of literature:

- **Gao et al. (2020)** studied that the exact pathogenesis of polycystic ovary syndrome (PCOS), the most common neuroendocrine disorder in women of reproductive age, has not been fully elucidated. Recent studies suggested that chronic inflammation and neurotransmitter disorder involved in the progress of PCOS. In this study, he investigated the possible protective effect and mechanism of troxerutin in a dihydrotestosterone (DHT) - induced rat model of PCOS. The PCOS rat models were treated with troxerutin at a dose of 150 mg/kg or 300mg/kg for up to 4 weeks. Results showed that 300 mg/kg troxerutin significantly decreased the body weight gain and improved the pathological changes of ovary induced by DHT. Meanwhile, the elevated gonadotrophin-releasing hormone (GnRH), gonadotrophin and testosterone in the serum of PCOS rats were reduced with the treatment of troxerutin Of note, the GnRH inhibitory regulator GABA and stimulatory regulator glutamate were also restored to the normal level by troxerutin. The present study indicated that troxerutin may exhibit a protective effect in PCOS rat model via regulating neurotransmitter release.
- **Angin et al., 2019** designed two questionnaire form of quality of life were given to 238 women, only 49 infertile PCOS patients, 47 infertile non-PCOS patients, and 62 fertile PCOS patients filled the forms. As per result Both PCOSQ and SF-36 scores are lowest in the infertile PCOS group. Although the total PCOSQ score of infertile non- PCOS group was better than non-infertile PCOS and fertile PCOS groups; there was a trend for lower scores when infertility is added on PCOS status.
- **Soni et. al. (2018)** characterized Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. It is characterized by hyperandrogenism, polycystic ovaries, and chronic anovulation along with insulin resistance, hyperinsulinemia, abdominal obesity, hypertension, and dyslipidemia as frequent metabolic traits (metabolic syndrome) that culminate in serious long-term consequences such as type 2

diabetes mellitus, endometrial hyperplasia, and coronary artery disease. It is one of the most common causes of anovulatory infertility. Pathogenesis of PCOS is explaining as alteration in gonadotropin-releasing hormone secretion results in increased luteinizing hormone (LH) secretion. An alteration in insulin secretion and insulin action results in hyperinsulinemia and insulin resistance. defect in androgen synthesis that results in increased ovarian androgen production. Treatment of PCOS include maintaining a normal endometrium, antagonizing the actions of androgens on target tissues, reducing insulin resistance when present), and correcting anovulation. Women with polycystic ovary syndrome (PCOS) are at higher risk for several other health conditions as Insulin Resistance, Metabolic Syndrome, Type 2 Diabetes, Obesity, Heart Disease and High Blood Pressure (Cardiovascular Disease).

- **Hector F et. al., (2018)** suggested that Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in premenopausal women. Heterogeneous by nature, PCOS is defined by a combination of signs and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnoses. The etiology of this syndrome remains largely unknown, but mounting evidence suggests that PCOS might be a complex multigenic disorder with strong epigenetic and environmental influences, including diet and lifestyle factors. PCOS is frequently associated with abdominal adiposity, insulin resistance, obesity, metabolic disorders, and cardiovascular risk factors. The diagnosis and treatment of PCOS are not complicated, requiring only the judicious application of a few well-standardized diagnostic methods and appropriate therapeutic approaches addressing hyperandrogenism, the consequences of ovarian dysfunction and the associated metabolic disorders.
- **Dawane J.S et. al., (2019)** Studied the efficacy of herbal formulation Niśāmalakīin animal model of polycystic ovarian disease syndrome. By using Six female rats of Group I served as control (No Letrozole,

no treatment), Letrozole was given 1mg/kg /day for 28 days to induce PCOS in remaining female Wistar rats which were then divided into 6 groups of 6 animals each Group II- PCOS disease control, Group III – Clomiphene citrate, Group IV- Nishamalaki, Group V- Metformin, Group VI- Nishamalaki & Metformin and Group VII- KanchanarGuggulu. Animals were treated orally according to groups for 56 days. Bodyweight, food intake, Insulin, blood glucose levels, and lipid profile checked before and after treatment. Vaginal smear was done daily to study estrous changes. As per study Rats with PCOS showed an increase in body weight and insulin levels. Significant reduction in body weight, lipid profile, Blood sugar, and insulin was seen in Nishamalaki treated animals but not in the KanchanarGuggulu group. KanchanarGuggulu and Nishamalaki regularized the cycles in 1st and 3rd weeks respectively.

- **Escobar-Morreale et. al., (2018),** H.F suggested that Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in premenopausal women. Heterogeneous by nature, PCOS is defined by a combination of signs and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnoses. The etiology of this syndrome remains largely unknown, but mounting evidence suggests that PCOS might be a complex multigenic disorder with strong epigenetic and environmental influences, including diet and lifestyle factors. PCOS is frequently associated with abdominal adiposity, insulin resistance, obesity, metabolic disorders, and cardiovascular risk factors. The diagnosis and treatment of PCOS are not complicated, requiring only the judicious application of a few well-standardized diagnostic methods and appropriate therapeutic approaches addressing hyperandrogenism, the consequences of ovarian dysfunction and the associated metabolic disorders.
- **Mohammad M.B et. al., (2017)** discussed that polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility in women of their reproductive age. It may also constitute a risk factor for cancer development. Different factors could result in

different manifestations and many of these are related to predispositions. It is essential to establish criteria to achieve an exact diagnosis of PCOS, especially among adolescent patients because of the overlap between features of PCO syndrome and physiological findings in puberty. The technology of ultrasonography is improving and accuracy is increasing but remains dependent on the specific equipment available. Some factors are inter-related in determining PCOS prognosis. Serum AMH is synthesized by small antral follicles, which are precisely those seen on ultrasound and could help us to diagnose PCOS but many aspects still require elucidation. In this mini-review, we have attempted to identify some of these correlations.

- **G. Morgante, et. al., (2017)** Studied that Polycystic ovary syndrome (PCOS) is a complex endocrine disorder affecting 5–10% of women of reproductive age. It generally shows with oligo/amenorrhea, anovulatory cycles, clinical o biochemical hirsutism, and polycystic ovaries and, in a significant percentage of cases, insulin resistance. PCOS is defined as a multifactorial pathology, determined by the association of many factors: genetic, endocrine and environmental. The first and most effective treatment of PCOS is to change life-style and lose weight. The use of oral contraceptives has been shown effective in reducing acne and hirsutism and regulates the menstrual cycle. For women with severe hirsutism, the addition of antiandrogens to estrogen–progestin therapy has significantly improved the results. In cases of anovulatory infertility, the drug of first choice is clomiphene citrate, followed by low-dose gonadotropins. Recently, insulin-sensitizing drugs have been widely prescribed for PCOS patients.
- **Cunningham P (2017)** Studied that PCOS is an incurable disorder of ovarian dysfunction. It is thought to be linked to an interaction between environmental and genetic factors and insulin resistance. During diagnosis, it is important to consider other conditions, such as potential neoplastic causes of androgen production, which may result in symptoms associated with PCOS. Emma's symptoms of

oligomenorrhoea, hyperandrogenism and the presence of polycystic ovaries confirmed by ultrasound scan, meant that she met the criteria for a diagnosis of PCOS. Pharmacological treatment available to patients with PCOS includes the combined oral contraceptive pill, which was prescribed for Emma by the practice nurse. Non-pharmacological treatment can also reduce the long-term risks of PCOS. PCOS can distressing for women and may affect their quality of life. It is important for nurses to provide individualized support to women undergoing treatment for the condition.

- **El Hayek, S., et al. (2016),** discussed that polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility in women of their reproductive age. It may also constitute a risk factor for cancer development. Different factors could result in different manifestations and many of these are related to predispositions. It is essential to establish criteria to achieve an exact diagnosis of PCOS, especially among adolescent patients because of the overlap between features of PCO syndrome and physiological findings in puberty. The technology of ultrasonography is improving and accuracy is increasing but remains dependent on the specific equipment available. Some factors are inter-related in determining PCOS prognosis. Serum AMH is synthesized by small antral follicles, which are precisely those seen on ultrasound and could help us to diagnose PCOS but many aspects still require elucidation. In this mini-review, we have attempted to identify some of these correlations.
- **Frank G et. al., (2015)** described that pathophysiology paradigm shift has emerged with the discovery that polycystic ovary syndrome is a proinflammatory state. Despite the dogma that the compensatory hyperinsulinemia of insulin resistance is the promoter of hyperandrogenism, physiological insulin infusion does not affect androgen levels in PCOS. The dogma also does not explain the cause of hyperandrogenism and ovarian dysfunction in the 30 to 50% of women with polycystic ovary syndrome who are of normal weight and lack insulin resistance. Inflammation is the underpinning of insulin resistance in obesity and type 2 diabetes, and may also be the

cause of insulin resistance when present in PCOS. The origin of inflammation in PCOS has been ascribed to excess abdominal adiposity or obesity. However, nutrients such as glucose and saturated fat can incite inflammation.

- **Meczekalski B. et. al., (2014)** Studied that FHA is an underestimated clinical problem. It is related to profound impairment of reproductive functions including anovulation and infertility. Women's health in this disorder is disturbed in several aspects including their skeletal system, cardiovascular system and mental problems. Patients manifest a decrease of bone mass density, which is related to an increase of fracture risk. Therefore, osteopenia and osteoporosis are the main long-term complications of FHA. Cardiovascular complications include endothelial dysfunction and abnormal changes in the lipid profile. FHA patients present significantly higher depression and anxiety and also sexual problems compared to healthy subjects. FHA patients should be carefully diagnosed and properly managed to prevent both short- and particularly longterm medical consequences.
- **Mark O.PCOS et. al., (2011)** suggested that is a major threat to women's health. Once considered a cosmetic or fertility disorder, PCOS is now recognized to carry considerable morbidity and long-term risk, particularly for glycemic abnormalities and T2DM. Other morbidities include CVD, obstetrical complications and mood and/or affective disorders. The pathogenesis of PCOS remains elusive, with contributions from insulin resistance, adipose tissue dysfunction, abnormal steroidogenesis and hypothalamic–pituitary–ovarian dysregulation, not all of which exist in any given patient. Genetic variants and epigenetic environmental factors, possibly beginning in utero, probably contribute to the dysregulation of these varied systems and raise new avenues of research investigation in the rapidly evolving field of PCOS.

Rationale:

As per the literature review, it was found that current drug for the treatment of anovulation infertility & FOH are potent but had a multiple effects. So, a new drug formulation is required with maximum potency and a target specific drug delivery system with at least negligible side effects. For the first time we are investigating the therapeutic role of apolipoprotein nanoparticles of norethisterone for treatment of Anovulation infertility & FOH associated with polycystic ovary Syndrome symptoms.

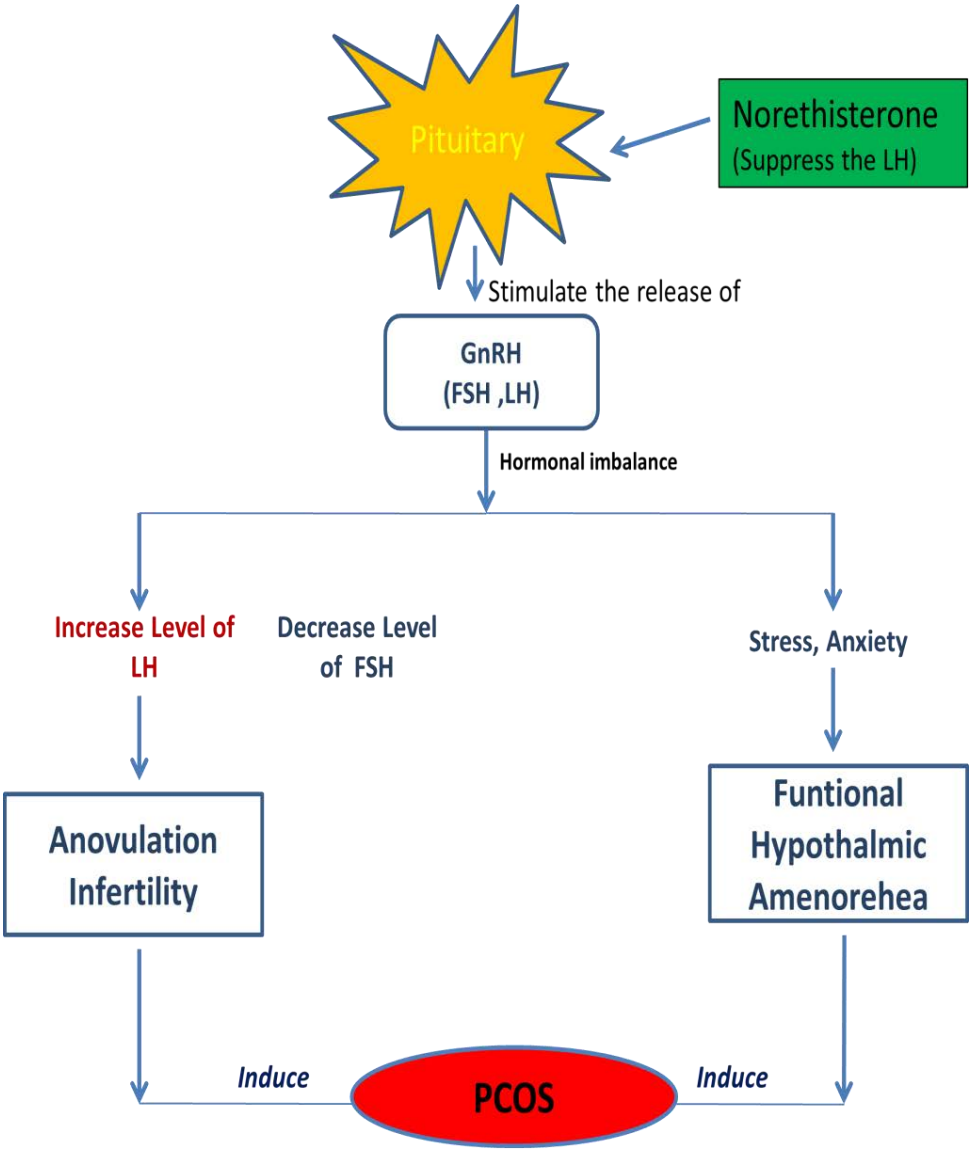
3. Aim of study:

Evaluation of pharmacological effect of apolipoprotein nanoparticles of norethisterone for anovulation infertility and functional hypothalamic amenorrhea associated with PCOS symptoms in female rats. Nanotechnology can be an important tool to improve the permeability of some drugs for the blood–brain barrier. In this work we created a new system to enter the brain by functionalizing solid lipid nanoparticles with apolipoprotein, aiming to enhance their binding to low-density lipoprotein receptors on the blood–brain barrier. The blood–brain barrier (BBB) is an almost impermeable, highly selective and dynamic specialized barrier system. Hereby, lipid-based nanocarriers hold strong promise to the delivery of drugs directly to the brain because of their lipid nature (lipophilicity) and small size, which gives the apolipoprotein a natural tendency to cross the BBB; their very low cytotoxicity, Therefore, in the present work, we will develop a novel approach to functionalize with Apo-lipoprotein in order to mediate the transport and uptake into the brain by a specific targeted drug delivery system and allow the whole nanocparicles. and the loaded drug to go through the BBB**.**

Objective of study:

- To carryout Preformulation study.
- Compatibility study between drug and excipients.
- To optimize the ratio of ingredients for successful formulation of apolipoproteins nanopaticles.
- To formulate apolipoproteins nanopaticles
- To evaluate the optimized formulation for various parameter.
- To check the stability of optimized formulation.
- To evaluate the optimized formulation for biological activity.
- To evaluate pharmacological effects the formulation.

4. Hypothesis:



5. Proposed Research Methodology:

1. **Literature survey:** The literature collects from the review papers, Google Scholar, PUBMED, MEDLINE, NCBI, Elsevier, Springer, and Science Direct.
2. **Selection of Drug:** As per the literature review, it was found that current drugs for the treatment of Anovulation infertility & FOH associated with PCOS symptoms are potent but had multiple effects. So, a new drug formulation is required with maximum potency and a target specific drug delivery system with at least negligible side effects. In the present study apolipoprotein nanoparticles of norethisterone will be prepare by using the targeted drug delivery system which are site specific (BBB) can be more effective then tablet formulation. Thus this study is designed to investigate the pharmacological effect of apolipoprotein nanoparticles of norethisterone for anovulation infertility and functional hypothalamic amenorrhea associated with PCOS symptoms in female rats. From the latest literature, it was found that till now no experimental work has been done on such formulation. So for the purpose of novelty for this study, I have selected this new formulation respectively [36].
3. **Selection of Models:**

1. DHT induced Anovulation infertility.
2. Letrozole induced FOH.

Animals:

Female Rats (n = 8 per group) in the weight range of 200—220 g will be used and the Animals will housed in standard plastic cages in a room maintained at temperature (24 ± 2∘ C) under 12/12-hr light-dark cycle. Food and water will provide ad libitum.

Drugs:

Norethisterone, DHT and letrazole will purchased from the market and dissolved in Soluble in chloroform and dioxan. All the doses will administer acutely in a volume of 5 ml/kg body weight of rats.

Induction of Anovulation infertility & FOH:

Postnatal 14 days female SD rats will be housed under controlled lighting (12 h light, 12 h darkness, lights and temperature (24 °C) with free access to food

and water for a week of acclimatization. Postnatal 21 days rats weighing approximately 50-55g and each rat of the disease group will be implanted with a silastic tube filled with 5α- DHT at dose of (1 mg) for 30 days [37].

1. PCOS:

Female Wistar rats will be housed under controlled lighting (12h light, 12 h darkness, lights and temperature (24 °C) with free access to food and water for a week of acclimatization. Each rat of the disease group will be received the letrozole 1mg/kg/day, po; for 21 days. [38].

3.Experimental Protocol: To evaluate the effect of this test substance on anovulation infertility, FOH associated with PCOS symptoms animals will be divided into 8 groups (n = 8 in each group) including Normal group, disease group (DHT), disease group (letrozole), Treatment group (low dose & high dose) in both disease groups & Standard drug group.

4. Table.1. Experimental Grouping:

1. Anovulation infertility:-

Groups	Species	Number of Animals
Normal control	Wistar rats (Female)	6
Disease (DHT:1mg/kg/day p.o : 30days)		6
Treatment (Norethisterone :0.5mg/day p.o: 5days)		6
Treatment (Norethisterone :1mg/day p.o : 5days)		6
Standard(Clomiphene Citrate :50mg/kg/day p o: 5days)		6

2. Functional hypothalamic amenorrhea:-

Groups	Species	Number of Animals
Normal control	SD rats (Female)	6
Disease (Letrozole:1mg/kg/day p.o : 21days)		6
Treatment (Norethisterone :0.5mg/day p.o: 5days)		6
Treatment (Norethisterone :1mg/day p.o : 5days)		6
Standard(Clomiphene Citrate :50 mg/kg/day p o: 5days)		6

Parameter to be assessed:-

During study protocol different parameters will be assessed:

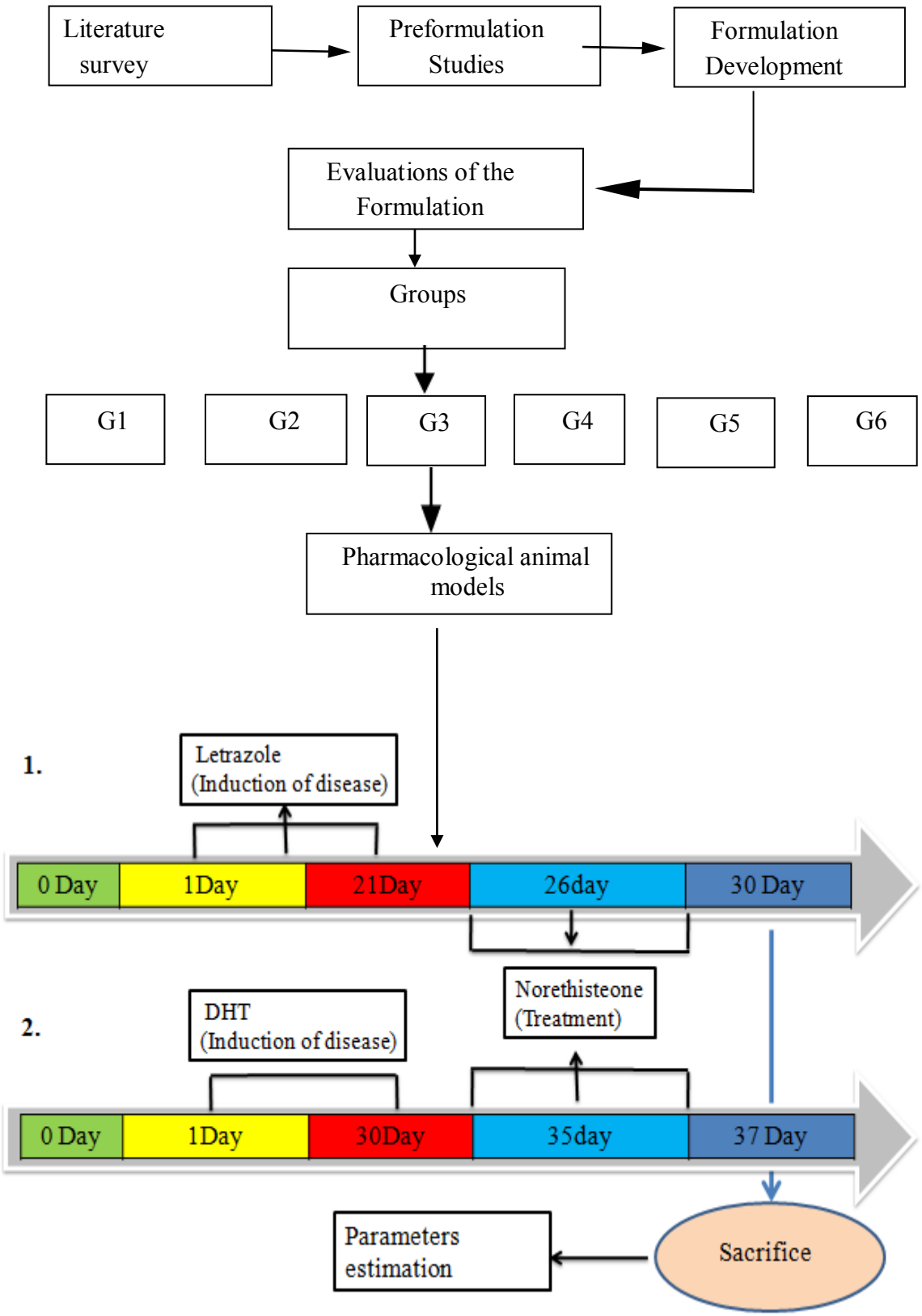
Table.2.Parameters to be assessed

S. No.	Parameters
1	**Physical Assesment:** ➢ Vagina smears and estrous cycles ➢ Body weight ➢ Weight of ovary & Uterus ➢ Mean arterial B.P
2	**Hemodynamic Assessments:** ➢ Histological assessment ➢ Blood Glucose level
3	**Biochemical analysis:** ➢ Serum Nitrite/Nitrate level ➢ Reduce Glutathione estimation
4	**Lipid Profile:** ➢ Cholesterol level ➢ HDL level ➢ Triglyceride level
5	**Hormonal Evaluation:** ➢ LH Level ➢ FSH Level ➢ Progesterone Level ➢ Estradiol Level
6	**Chromatographic analysis:** ➢ GABA Level ➢ Glutamate Level

5. Statistical Analysis of data.

All data will be expressed with the help of Statistical Software Graph Pad Prism-8.

6. Tentative chapter plan:



7. References:

1.Weiss, R.V. and R. Clapauch, *Female infertility of endocrine origin.* Arquivos Brasileiros de Endocrinologia & Metabologia, 2014. **58**: p. 144-152.

2. Berga, S., et al., *Neuroendocrine aberrations in women with functional hypothalamic amenorrhea.* The Journal of Clinical Endocrinology & Metabolism, 1989. **68**(2): p. 301-308.

3. Gordon, C.M., *Functional hypothalamic amenorrhea.* New England Journal of Medicine, 2010. **363**(4): p. 365-371.

4. Genazzani, A.D., *Neuroendocrine aspects of amenorrhea related to stress.* Pediatric endocrinology reviews: PER, 2005. **2**(4): p. 661-668.

5. Meczekalski, B., et al., *Functional hypothalamic amenorrhea: current view on neuroendocrine aberrations.* Gynecological endocrinology, 2008. **24**(1): p. 4-11.

6. Harlow, S.D., G. Windham, and P. Paramsothy, *Menstruation and menstrual disorders*, in *Women and health*. 2000.

7.Berga, S.L., T.L. Daniels, and D.E. Giles, *Women with functional hypothalamic amenorrhea but not other forms of anovulation display amplified cortisol concentrations.* Fertility and sterility, 1997. **67**(6): p. 1024-1030.

8.Valdes-Socin, H., et al., *Reproduction, smell, and neurodevelopmental disorders: genetic defects in different hypogonadotropic hypogonadal syndromes.* Frontiers in endocrinology, 2014. **5**: p. 109.

9.Laughlin, G.A., C. Dominguez, and S. Yen, *Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea.* The journal of clinical endocrinology & Metabolism, 1998. **83**(1): p. 25-32.

10.Gordon, C.M., et al., *Functional hypothalamic amenorrhea: an endocrine society clinical practice guideline.* The Journal of Clinical Endocrinology & Metabolism, 2017. **102**(5): p. 1413-1439.

11.Genazzani, A., et al. *Hypothalamic amenorrhea: from diagnosis to therapeutical approach*. in *Annales D'endocrinologie*. 2010. Elsevier.

12.Prokai, D. and S.L. Berga, *Neuroprotection via reduction in stress: altered menstrual patterns as a marker for stress and implications for long-term neurologic health in women.* International journal of molecular sciences, 2016. **17**(12): p. 2147.

13.Rafique, N. and M.H. Al-Sheikh, *Prevalence of menstrual problems and their association with psychological stress in young female students studying health*

sciences. Saudi medical journal, 2018. **39**(1): p. 67.

14.Schneiderman, N., G. Ironson, and S.D. Siegel, *Stress and health: psychological, behavioral, and biological determinants.* Annu. Rev. Clin. Psychol., 2005. **1**: p. 607-628.

15.Petraglia, F., et al., *Opioid control of LH secretion in humans: menstrual cycle, menopause and aging reduce effect of naloxone but not of morphine.* Life sciences, 1986. **38**(23): p. 2103-2110.

16. Liu, J.H. and A.H. Bill, *Stress-Associated or Functional Hypothalamic Amenorrhea in the Adolescent.* Annals of the New York Academy of Sciences, 2008. **1135**(1): p. 179-184.

17. Thackray, V.G., P.L. Mellon, and D. Coss, *Hormones in synergy: regulation of the pituitary gonadotropin genes.* Molecular and cellular endocrinology, 2010. **314**(2): p. 192-203.

18. Amsterdam, A., et al., *Mechanisms of gonadotropin desensitization.* Molecular and cellular endocrinology, 2002. **187**(1-2): p. 69-74.

19. Hai, T. and M.G. Hartman, *The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis.* Gene, 2001. **273**(1): p. 1-11.

20. Ortmann, O., J. Weiss, and K. Diedrich, *Gonadotrophin-releasing hormone (GnRH) and GnRH agonists: mechanisms of action.* Reproductive biomedicine online, 2002. **5**: p. 1-7.

21. Huirne, J.A. and C.B. Lambalk, *Gonadotropin-releasing-hormone-receptor antagonists.* The Lancet, 2001. **358**(9295): p. 1793-1803.

22. Norman, R.J., et al., *Polycystic ovary syndrome.* The Lancet, 2007. **370**(9588): p. 685-697.

23. Allahbadia, G.N. and R. Merchant. *Polycystic ovary syndrome in the Indian Subcontinent*. in *Seminars in reproductive medicine*. 2008. © Thieme Medical Publishers.

24. El Hayek, S., et al., *Poly cystic ovarian syndrome: an updated overview.* Frontiers in physiology, 2016. **7**: p. 124.

25. Soni, A., S. Singla, and S. Goyal, *Polycystic ovary syndrome: pathogenesis, treatment and secondary associated diseases.* Journal of Drug Delivery and Therapeutics, 2018. **8**(5): p. 107-112.

26. Yildiz, B.O., *Recent advances in the treatment of polycystic ovary syndrome.* Expert opinion on investigational drugs, 2004. **13**(10): p. 1295-1305.

27. Smith, C., et al., *British Association of Dermatologists guidelines for biologic therapy for psoriasis 2017.* British Journal of Dermatology, 2017. **177**(3): p. 628-636.

28. Van Onselen, J., *Hirsutism: causes and treatment for women.* British Journal of Nursing, 2011. **20**(16): p. 985-990.

29. Cunningham, P., *Pathophysiology, diagnosis and treatment of polycystic ovary syndrome.* Nursing Standard, 2017. **31**(39).

30. Badawy, A. and A. Elnashar, *Treatment options for polycystic ovary syndrome.* International journal of women's health, 2011. **3**: p. 25.

31. Soni, A., *Development of intravaginal liposomal gel of Clomiphene citrate for treatment of polycystic ovary syndrome.* 2019.

32. Soni, A., et al., *UV spectrophotometric analytical method development and validation of clomiphene citrate in methanol.* Int. J. Pharm. Sci. & Res., 2020. **11**: p. 2229-2237.

33. Ghahiri, A., N. Mogharehabed, and M. Mamourian, *Letrozole as the first-line treatment of infertile women with poly cystic ovarian syndrome (PCOS) compared with clomiphene citrate: A clinical trial.* Advanced biomedical research, 2016. **5**.

34. Stratakis, C.A., et al., *Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease.* Annals of internal medicine, 1999. **131**(8): p. 585-591.

35. Siriwardene, S.D., et al., *Clinical efficacy of Ayurveda treatment regimen on Subfertility with poly cystic ovarian syndrome (PCOS).* Ayu, 2010. **31**(1): p. 24.

36. Escobar-Morreale, H.F., *Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment.* Nature Reviews Endocrinology, 2018. **14**(5): p. 270.

37. Walters, K.A., C.M. Allan, and D.J. Handelsman, *Rodent models for human polycystic ovary syndrome.* Biology of reproduction, 2012. **86**(5): p. 149, 1-12.

38. Rajan, R.K. and B. Balaji, *Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (PCOS) model through anti-androgenic mechanism.* Pharmaceutical biology, 2017. **55**(1): p. 242-251.

Signature of Candidate **Signature of Supervisor**